

ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF LETTUCE

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Abstract

In recent years, lettuce has been increasingly used in human diet throughout the year because of its nutritional and medicinal properties. In this research, lettuce *Lactuca sativa* L. var. *romana* (marula) was used. The content of the antioxidant compounds (phenolic compounds, L-ascorbic acid, β -carotene and lycopene) and the antioxidant activity were determined in ethanolic extracts of the lettuce by means of spectrophotometric methods. A high content of phenolic components provides favourable antioxidant properties found in the examined lettuce. According to the results, the lettuce extract displays the antioxidant activity, with the total antioxidant capacity of 78.98 ± 0.25 μg of ascorbic acid/g and 50% inhibition concentration values of 26.95 ± 0.99 $\mu\text{g/mL}$ for 2,2-diphenyl-1-picrylhydrazyl free radical scavenging activity, and 98.88 ± 0.94 $\mu\text{g/mL}$ for hydroxyl radical scavenging activity.

The antimicrobial activity of the lettuce extract, was tested with bacteria from clean cultures *Staphylococcus aureus* ATCC 25923, *Klebsiella pneumoniae* ATCC13883, *Escherichia coli* ATCC 25922, *Proteus vulgaris* ATCC13315, *Proteus mirabilis* ATCC14153, *Bacillus subtilis* ATCC6633, and fungi *Candida albicans* ATCC10231 and *Aspergillus niger* ATCC16404. The antimicrobial activity was determined by microdilution method (MIC). The smallest susceptibility to the ethanolic extract of lettuce was exhibited by the bacteria *Staphylococcus aureus* and *Proteus vulgaris* (MIC=78,125 $\mu\text{g/ml}$), while the other selected bacteria and fungi showed higher susceptibility (MIC=39,1 $\mu\text{g/ml}$).

Keywords: lettuce, antioxidant activity, antimicrobial activity

Introduction

Vegetables and fruits are rich sources of antioxidants such as vitamins A, C and E, carotenoids, polyphenolic components, and flavonoids (Mladenovic et al. 2011), which prevent the attack of free radicals thus reducing the risk of carcinogenic illnesses. Consumption of antioxidants in food via natural sources is good for the prevention of cardiovascular diseases, especially arteriosclerosis (Hu 2000).

Lettuce (*Lactuca sativa* L.), an annual plant, belongs to the family *Asteraceae* and is a very significant leaf vegetable which is primarily consumed fresh as a salad or in salad mixtures with other kinds of fresh vegetables. It is the most popular leaf vegetable and is consumed in increasingly greater amounts owing to the realizations regarding its nutritive values (Dupont et al. 2000), as well as the fact that it is used fresh so that all the ingredients remain intact. Lettuce is used almost throughout the year since there are a number of varieties which are successfully cultivated in early spring, during the summer and winter. In everyday nutrition lettuce is of great significance primarily for the amount of biologically active substances it contains, especially phenolic compounds, ascorbic acid, vitamins A and K, folates and carotenoids (Llorach et al. 2008). The nutritive content varies depending on the lettuce type (Mou 2005). The leaf types (Cos and Cutting)

have a significant amount of ascorbic acid, vitamins A and K, folates, carotenoids and β -carotene, lutein and zeaxanthin. These types are especially rich in vitamin A and β -carotene, the amount of which is approximately 15 times larger than the one in the head-forming types of lettuce Crisphead type (Cooper 2004).

Recent research has shown that the ethanol extracts of lettuce possess an antimicrobial activity towards certain strains of microorganisms (Pavlovic et al. 2011).

In this paper the research has been performed on the lettuce *var. romana* (marula). A characteristic of this lettuce type is that it grows upright, has thick, firm, tasty leaves forming a shape of heart. It grows slower than butterhead and crisphead lettuce types. It succeeds better in cold conditions, on wet ground rich in humus. It is more resistant to frost than most lettuce types, so that certain strains are cultivated in winter in the open.

Materials and Methods

Sample preparation

Green leaves of the lettuce were extracted by 96% ethanol in the process of cold maceration. The ethanol was removed by a rotary evaporator (Devarot, Elektromedicina, Ljubljana, Slovenija) under a vacuum and was dried at 40°C. The dried extracts were stored in glass bottles at 4 °C to prevent oxidative damage until the analysis.

Spectrophotometric methods

Total phenols in the lettuce ethanol extracts were estimated according to the Folin–Ciocalteu method (Singleton et al. 1999). The absorbance was measured at 765 nm with a spectrophotometer against a blank sample. Gallic acid (GA) was used to calculate the standard curve. The assays were performed in triplicate; the results were the mean values \pm standard deviations and expressed as milligrams of gallic acid equivalents per gram of dry extract (mg of GA/g).

The aluminium chloride colorimetric method (Brighente et al. 2007) was used to measure the flavonoids content of the lettuce extracts. Rutin was used as a standard for the calibration curve. The estimation of the total flavonoids was performed in triplicate. The results were the mean values \pm standard deviations and expressed as rutin equivalents (mg of RU/g of dry extract).

The total antioxidant capacity of the examined lettuce extract was evaluated by the phosphomolybdenum method (Prieto et al. 1999). The assay is based on the reduction of Mo(VI) to Mo(V) by antioxidant compounds and subsequent formation of a green phosphate/Mo(V) complex at acid pH. Ascorbic acid (AA) was used as the standard, and the total antioxidant capacity was expressed as micrograms of AA per gram of dry extract (μ g AA/g dry extract).

The capacity to scavenge the “stable” free radical DPPH was monitored according to the method of Takao *et al.* (1994) adopted with suitable modifications from Kumarasamy *et al.* (2007). The 50% inhibition concentration (IC₅₀) value, defined as the concentration of the test material that leads to 50% reduction of the free radical concentration, was calculated as micrograms per millilitre through a sigmoidal dose-response curve.

The ability of the examined lettuce to inhibit a non-site specific hydroxyl radical-mediated peroxidation was carried out according to the method described by Hinneburg *et al.* (2006). The percentage inhibition values were calculated from the absorbance of the control and of the sample, where the controls contained all the reaction reagents except the extract or positive control substance.

Ascorbic acid was determined according to the method of Klein and Perry (1982). The content of ascorbic acid was calculated on the basis of the calibration curve of standard L-ascorbic acid (0.020–0.12 mg/ml). The results were expressed as milligrams of ascorbic acid/100 g of fresh lettuce.

-Carotene and lycopene were determined according to the method of Nagata and Yamashita (1992). The absorbance was measured at 453, 505, 645 and 663 nm. Contents of β -carotene and lycopene were calculated according to the following equations:

$$\text{lycopene (mg/100 ml)} = -0.0458 A_{663} + 0,204 A_{645} + 0.372 A_{505} - 0.0806 A_{453};$$

$$\beta\text{-carotene (mg/100 ml)} = 0.216 A_{663} - 1,22 A_{645} - 0.304 A_{505} + 0.452 A_{453}.$$

Microdilution Method (MIC)

The minimum inhibitory concentrations (MIC) of the lettuce extract against the tested bacteria and fungi were determined using a microdilution method in 96 multi-well microtiter plates (Satyajit et al. 2007). All tests were performed in Muller–Hinton broth (MHB), with the exception of yeast, in which case Sabouraud dextrose broth was used. Growing conditions and media sterility have been checked for each strain. A standard antibiotic, “Amracin,” has been used for controlling the sensitivity of the examined bacteria, while “Nystatin“ has been used as a control for the tested fungi. As an indicator the solution resazurin has been used, and it has been added in each well. After the incubation (on 37 °C for 24h) the minimal inhibiting concentration has been determined visually, on the basis of colour. Each change of colour from purple to pink or clear has been considered to be positive. The lowest concentration that caused the changing of colour has been taken as MIC value. Material has been measured three times and the calculated mean value has been taken for MIC.

Results and Discussion

The results of the analysis of the antioxidant compounds content and the antioxidant activity of *Lactuca sativa* L. var. *Romana* extract are given in Table 1. The obtained values show that the ethanolic extract of the examined lettuce contains a high concentration of the total phenols and flavonoids. Owing to the presence of these compounds the antioxidant capacity is somewhat higher than in the earlier examined varieties of lettuce (Zdravkovic J. et al. 2013). Phenolic compounds and flavonoids have been reported to be associated with the antioxidant action in biological systems, mainly due to their reduction–oxidation properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (Saha et al. 2008). However, the ethanolic extract of lettuce *Romana* $IC_{50} = 26,95 \pm 0,99$ $\mu\text{g/ml}$ showed less antioxidant activity of the ethanolic extract of, for instance, AA $IC_{50} = 10.61\mu\text{g/ml}$ and BHT 39.25 $\mu\text{g/ml}$. We could say that the amount of the other antioxidant compounds (L-ascorbic acid, β -carotene and lycopene) remains within the boundaries recorded in other lettuce types (Llorach et al. 2008).

Table 1. The Content of the Antioxidant Compounds and the Antioxidant Activity of the tested *Lactuca sativa* L. var *Romana* Extract

Compound	Value
Total phenolics (mg of GA/g)	79,54 ± 0,43
Flavonoids (mg of RU/g)	27,98 ± 0,88
Total antioxidant capacity (µg of AA/g)	78,98 ± 0,25
DPPH, IC ₅₀ (µg /ml)	26,95 ± 0,99
HRSA, IC ₅₀ (µg /ml)	98,88 ± 0,94
L-ascorbic acid (mg /100 g)	6,80 ± 0,35
-carotene (mg /100 g)	3,40 ± 0,28
lycopene (mg /100 g)	0,40 ± 0,03

GA, gallic acid; RU, rutin, AA, ascorbic acid;

DPPH, 2,2-diphenyl-1-picrylhydrazyl free radical scavenging activity;

HRSA, hydroxyl radical scavenging activity.

Table 2. Minimum Inhibitory Concentrations of the Ethanolic Extract of *Lactuca sativa* L. var. *Romana* and the Standard Drugs for Eight Indicator Strains

Microbial strains	MIC (µg /ml)		
	<i>Romana</i> ethanolic extract	Amracin	Nystatin
<i>S. aureus</i> ATCC 25923	78,12	0,97	/
<i>K. pneumoniae</i> ATCC 13883	39,10	0,49	/
<i>E. coli</i> ATCC 25922	39,10	0,97	/
<i>P. vulgaris</i> ATCC 13315	78,12	0,49	/
<i>P. mirabilis</i> ATCC 14153	39,10	0,49	/
<i>B. subtilis</i> ATCC 6633	39,10	0,24	/
<i>C. albicans</i> ATCC 10231	39,10	/	1,95
<i>A. niger</i> ATCC 16404	39,10	/	0,97

The results of the analysis of the antimicrobial activity obtained by the dilution method are given in Table 2; MICs were determined for eight selected indicator strains. The results presented in Table 2 reveal antimicrobial activity of the ethanolic extract of *Lactuca sativa* L. var *Romana* within the concentration range of 39,10 µg/ml to 78,12 µg/ml. Equal susceptibility to the ethanolic extract of the lettuce among the strains tested was exhibited by *K. pneumoniae* ATCC 13883, *E. coli* ATCC 25922, *P. mirabilis* ATCC 14153, *B. subtilis* ATCC 6633, *C. albicans* ATCC 10231 and *A. niger*

ATCC 16404 (MIC= 39,10 µg/mL), and somewhat lower by *S. aureus* ATCC 25923 and *P. vulgaris* ATCC 13315 (MIC= 78,12 µg/mL).

Conclusion

On the basis of the obtained results, it can be concluded that the lettuce *Lactuca sativa* L. var *Romana* displays a pronounced antioxidant activity owing to the presence of the antioxidant compounds. The amount of the antioxidant components (phenolic compounds, L-ascorbic acid, β-carotene and lycopene) lies within the boundaries recorded in other lettuce types. The ethanolic extract of the examined lettuce showed a favourable antimicrobial activity when applied *in vitro* against the tested bacteria and fungi. These experiments confirmed that lettuce is necessary in human diet as a source of antioxidant components and vitamins, especially since it is used raw so that its nutritive value remains preserved. By comparing it to other lettuce types, it has been concluded that the amount of the examined antioxidant components depends on the genotype of lettuce, which gives an opportunity to selectors to work on the improvement of certain characteristics of new lettuce genotypes.

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References

- Brighente I.M.C., Dias M., Verdi L.G., Pizzolatti M.G. (2007): Antioxidant activity and total phenolic content of some Brazilian species, *Pharm. Biol.* 45: 156–161.
- Cooper D.A.(2004): Carotenoids in health and disease, Recent scientific evaluations, research recommendations and consumer. *J Nutr.* 134: 221S-224S.
- Dupont S., Mondi Z., Williamson G., Price K. (2000): Effect of variety, processing, and storage on the flavonoid glycoside and composition of lettuce and chicory, *Journal of Agricultural and Food Chemistry* 48: 3957–3964.
- Hinneburg I., Dorman H.J.D., Hiltunen R. (2006): Antioxidant activities of extracts from selected culinary herbs and spices, *Food Chem* 97: 122–129.
- Hu F.B.(2000):Dietary pattern analysis: a new direction in nutritional epidemiology.*Current Opinion in Lipidology*, 13: 3-9.
- Klein B. P., Perry A. K., (1982): Ascorbic acid and vitamin A activity in selected vegetables from different geographical areas of the United States, *Journal of Food Science* 47: 941–945.
- Llorach R., Martinez-Sanchez A., Tomas-Barberan F.A., (2008): Characterisation of polyphenols and antioxidant properties of five lettuce varieties and ascarole, *Food Chemistry A* 108: 1028-1038.
- Kumarasamy Y.,Byres M., Cox P.J., Jaspars M., Nahar L., Sarker S.D., (2007): Screening seeds of some Scottish plants for free-radical scavenging activity, *Phytother. Res.* 21: 615–621.
- Mladenovi J., Maškovi P., Pavlovi R., Radovanovi B., AČamovi - okovi G., Cvijovi M.(2011): Antioxidant activity of ultrasonic extracts of leek *Allium porrum* L. *Hemijska industrija* 65: (4) 473-477.
- Mou B. (2005): Genetic variation of beta-carotene and lutein contents in lettuce. *J. Am.Soc.Hort Sci.*, 130: 870-876.

- Nagata M. and Yamashita I., (1992): Simple method for simultaneous determination of chlorophyll and carotenoids in tomato fruit, *Nippon Shokuhin Kogyo Gakkaishi* 39: 925–928.
- Pavlovi R., Maskovi P., Mladenovi J., Zdravkovi J., AČamovi - okovi G., Zdravkovi M., Cviki D., (2011): In Vitro Antimicrobial Activity of Ethanol Lettuce Extracts as a Potential Natural Conservancy, 22nd International Symposium “Safe Food Production” Proceedings, Novi Sad, 417-419.
- Prieto P., Pineda M., Aguilar M., (1999): Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E, *Anal. Biochem.* 269: 337–341.
- Saha M. R., Hasan S. M. R., Akter R., Hossain M. M., Alam M. S., Alam M. A., Mazumder M. E. H., (2008): In vitro free radical scavenging activity of methanol extract of the leaves of *Mimusops elengi* Linn. *Bangladesh J Vet Med.* 6:197–202.
- Satyajit D. S., Lutfun N., Yashodharan K., (2007): Microtitre plate-based antibacterial assay incorporating resazurin as indicator of cell growth, and its application in the *in vitro* antibacterial screening of phytochemicals, *Methods.* 42(4): 321-324.
- Singleton V., Orthofer R., Lamuela-Raventos R.M., (1999): Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent, *Methods Enzymol* 299: 152–175.
- Takao T., Watanabe N., Yagi I., Sakata K., (1994): A simple screening method for antioxidants and isolation of several antioxidants produced by marine bacteria from fish and shellfish, *Biosci. Biotechnol. Biochem.* 58: 1780–1783.
- Zdravkovi J., AČamovi - okovi G., Mladenovi J., Pavlovi R., Zdravkovi M.: Antioxidant capacity and contents of phenols, ascorbic acid, β -carotene and lycopene in lettuce, <http://www.doiserbia.nb.rs/issue.aspx?issueid=1868>, Accessed on 17/6/2013.